Reviewer’s report

**Title:** Anti-inflammatory effects in muscle injury by transdermal application of gel with Lychnophora pinaster aerial part using phonophoresis in rats

**Version:** 2  **Date:** 17 August 2013

**Reviewer:** Norberto P Lopes

**Reviewer’s report:**

Dear Editor:

The work titled “Anti-inflammatory effects in muscle injury by transdermal application of gel with Lychnophora pinaster aerial part using phonophoresis in rats” has scientific merit, since it presents the anti-inflammatory activity of this species in model of muscle injury.

The methodologies used are appropriate to the goals, as well as, the results are well presented and discussed. The results presented in the work should be considered for publication. The interpretation of the data confirms the anti-inflammatory potential of the extracts and chemical constituents isolated from L. pinaster. The discussion of the data is adequate.

Decision: Accept the article for publication, after minor revisions.

Suggestions to authors:

Title: write “aerial parts”.

Abstract

Page 2:

The Background section should be written illustrating the research and its aims. Add information about popular use of the plant as an anti-inflammatory agent. The section should end with a brief description of what is being reported in the article. Re-write this section.

In the conclusion section of the abstract, change quercetin attenuates the inflammatory by “quercetin attenuates the inflammation”.

Background

Page 3:

The authors affirm that “This species exhibits antitumor, antimicrobial, anti-pyretic, analgesic, anticonvulsant, anti-inflammatory and antioxidant activities”. My question for the authors is: these activities are attributed specifically to L. pinaster or to species of the genus Lychnophora in general?
Methods
Page 5:
Plant material: write the word Herbarium without italic

Page 6:
Fractionation of aqueous extract (WE): in lines 3 and 5 of this section write “3:1 ethyl acetate:ethyl ether solution”.

In this section, the authors affirm that “the aqueous phase provided the alkaloid fraction”. The phytochemistry of this genus is well characterized by the presence of caffeoylquinic acid derivatives, flavonoids, sesquiterpenes and sesquiterpene lactones. To our knowledge, there is no occurrence of alkaloids in the genus Lychnophora. If there are reports, the authors should cite this work and then explain how it was made to characterize the presence of alkaloids in this fraction. It was used some reagent of identification (Dragendorff for example) or some other chromogenic agent? I wait for a response.

Write: “(1:4 methanol:water solution)

The numeration of the structure of quercetin in Figure 1 is not correct. The B ring of this flavonoid has a catechol nucleus, which is 3’-4’ dihidroxibenzenes. Thus, the correct numeration of the B ring in this structure is:

In page 6 change the hydrogens H-6’ by H-2’; H-2’ by H-6’ and H-3’ by H-5’. In this form of numeration, the chemical shifts of the carbons presented in the paper are correct.

Page 8:
Groups of rats (n= 36). The total number of animals is 36 per group (360 animals were used in total?) or 36 animals in all study? What is the number of animals per group?

Results and discussion
Page 11:
“The low inflammatory activity suggested for HE may be related to a low concentration of triterpenes in this extract, mainly the triterpene lupeol, which exhibits significant activity”. Inflammatory of anti-inflammatory activity???

Figure 1:
Correct the structure of quercetin and add the number (4) below the structure of 3-O-acetyl-lupeol. Correct structures 4 and 3, add the group acetyl with

Level of interest: An article of importance in its field

Quality of written English: Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.